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NUTRITION HIGHLIGHTS — 1950

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NUTRITION HIGHLIGHTS — 1950

By RUTH WOODS

During 1950, nutrition research and biochemistry have become more closely allied than ever. New discoveries in nutrition have been matched almost equally with new findings along biochemical lines. These combined researches have attempted to explain the mysterious behavior of foods and their component elements in the living organism and, in many cases, have come tantalizingly close to the heart of the matter. Still, there are many loose ends and elusive will o' the wisps to be grasped and pinned down, further examined and accurately fitted into the complex pattern of nutrition dynamics. Progress in this direction during the past year, however, has been such as to warrant a well-justified feeling of optimism that we are standing at the very threshold of final revelation in more than one field of nutrition research.

Prominent among these nutritional investigations have been studies amplifying the underlying nature of the anemias and of the common degenerative diseases of our times — hypertension, arteriosclerosis, diabetes, liver and kidney damage and carcinoma. These studies, along with advances in intravenous feeding during periods of medical emergency (before and after surgery, during coma and shock) re-emphasize the close dependance of medicine upon nutrition. Basic research studies have uncovered new information concerning the interdependence of nutrients; the significance of dietary unbalances; the relation of nutrition to soil, agriculture and livestock feeding; the role of nutrition in maternal and child health, in dental caries, in conditions of climatic stress (heat, cold, altitude). The discovery that antibiotics stimulate growth may afford a means of unravelling fundamental problems in both nutrition and medicine. Development of new research tools (analytical methods, micromethods, procedures for syntheses, isolation and separation of nutrients) have hastened considerably the progress of research.

In the brief space here allotted only a few of the many outstanding researches of the past year can be described; we have selected those which may be of especial interest to the readers of this periodical.

Folinic Acid and the Citrovorum Factor:

The use of the microorganism *Leuconostoc citrovorum* for microbiological assays in 1948 led to the discovery of a new vitamin factor closely

allied to folic acid. In the two years that followed considerable study of the citrovorum factor (CF) has been made by several groups of investigators, culminating last year in evidence elucidating its relation to folic acid (folacin) and establishing its probable identity with a similar factor described as "folinic acid." The citrovorum factor present in refined liver extracts is required by the aforementioned microorganism for growth. Both folic acid and thymidine* promote growth in this organism under the same conditions, but to a much lesser degree than does CF-containing liver extract. Studies demonstrating the ability of CF to overcome the inhibitory effects of aminopterin, a folic acid antagonist, further substantiated the proposed relationship between CF and folic acid. In 1949, "folinic acid" another substance or group of substances found to be effective in counteracting anti-vitamins of the folic acid group was reported to behave similarly to CF with respect to its growth-stimulating properties for *Leuconostoc citrovorum*. On the basis of chemical studies folinic acid was shown to be either very closely related to, or actually identical with, the citrovorum factor.

During 1950, the relationship between CF and folic acid has been greatly clarified by several investigators. Moreover, an interesting interrelation between both of these factors and vitamin C has been uncovered as well. Experimental investigation revealed that folic acid may be converted to the more active citrovorum factor both by liver slices and by chemical treatment. In both instances the conversion was remarkably accelerated by the presence of ascorbic acid. The chemically produced derivative exhibited a growth-promoting activity for *Leuconostoc citrovorum* about one million times as active as folic acid on a weight basis.

These studies serve to reemphasize the mutual interrelationship demonstrated for folic acid and ascorbic acid in studies on the metabolism of the aromatic amino acids in scorbutic guinea pigs† and in studies with folic acid-deficient rats. Folic acid and ascorbic acid have been used interchangeably in these instances to correct the existing deficiencies. The role of ascorbic acid in accelerating the conversion of folic acid to the citrovorum factor or to folinic acid (which conceivably may be the form in which folic acid is required for the performance of its various biological functions) may be one explanation of the observed interrelations between folic acid and vitamin C.

**cf. Borden's Review of Nutrition Research,* Nov. 1949, "Recent Advances in the Knowledge of Anti-Anemia Factors."

†*cf. Borden's Review of Nutrition Research,* Sept. 1949, "Dietary Interrelationships: Vitamin C and the Metabolism of Aromatic Amino Acids."

Antibiotics and Growth:

During the course of studies on vitamin B_{12} and other possible components of the animal protein factor complex, the observation was made that animals frequently grew faster when given crude B_{12} concentrates than when given the pure crystalline vitamin. The concentrates supplying the vitamin were derived from mold fermentation mashes used in the production of antibiotics.

Accordingly, the pure antibiotics were fed, both separately and in conjunction with the pure vitamin B_{12}. Results of several groups of investigators indicated that far greater growth resulted when crystalline aureomycin, terramycin and other antibiotics were fed than has ever been obtained on diets providing adequate amounts of all the known vitamins. In feeding studies with chickens, pigs and turkeys, results similar to those with laboratory animals were obtained, many investigators reporting weight gains of the order of 30 per cent above expected performance on customary well-balanced rations. A considerable number of theories have been advanced to account for this unexpected behavior of the antibiotics, but none of these has as yet been confirmed. Prolonged feeding tests indicate no harm resulting from the feeding of low levels of antibiotics. Although the mode of action of these compounds remains undiscovered as yet, this newest nutritional discovery has already found practical application in the form of recently introduced commercial animal feeds fortified with suitable levels of one or more of the antibiotics.

Recent Advances in Cardiovascular Disease:

The nutritional approach to the control of cardiovascular disease — notably high blood pressure and hardening of the arteries — has been most promising. During 1950, several noteworthy findings have been reported. The relation of short periods of severe choline deprivation during the period of early growth in animals to irreversible changes in the heart and circulatory system resembling those of human cardiovascular disease has been confirmed and further extended. The efficacy of diets low in protein, calories and salt in restoring elevated blood pressures to normal levels in animals and in human subjects as well has been affirmed. The detection, by ultra-centrifugal examination of the blood, of giant cholesterol-containing molecules of abnormal character has provided a partial explanation of the role of cholesterol in the development of arteriosclerosis and promises to be of great potential value as a diagnostic aid in revealing existing or incipient

hardening of the arteries before clinical symptoms become evident.* The discovery that, under certain conditions, the vitamin, inositol, will lower the blood level of cholesterol promises to be of value in the clinical consideration of arteriosclerosis and other conditions associated with high blood levels of cholesterol.

New Concept of Transmethylation:

Indications of a relationship between vitamin B_{12} and the so-called biological methylating agents (choline, methionine, betaine) described during 1949,† led to a reinvestigation of the phenomenon of transmethylation in the light of new findings made possible following the discovery of vitamin B_{12}. During 1950 a number of studies were made which have served to amplify the original concept of transmethylation and to shed further light on the mechanics of its operation. The concept of transmethylation originally proposed the need for preformed methyl groups in the diet, possessing the ability of being transferred from one compound to another to form essential metabolites from precursors existing in the diet or in the body. While the basic principles of the transmethylation reaction remain valid, the mechanisms whereby these reactions are believed to occur have been considerably elucidated and entail several modifications of the original theory. Three phases of this phenomenon merit attention in the light of findings of the past year:

1. Methyl donors have been shown to have a high degree of specificity. In other words, compounds containing "labile" methyl groups are not all equivalent, but each methyl donor may have its own specific methyl acceptor. Moreover, each of these individual reactions is catalyzed by a specific enzyme system. Vitamin B_{12} may possibly function as part of at least one such enzyme system.
2. "Labile" methyl groups may not only be transferred intact, but may also undergo biochemical degradation and resynthesis during transfer from one compound to another.
3. The essentiality of methyl groups in the diet is a matter of degree, depending on environmental conditions (much as in the sense of the "essential" *vs.* "non-essential" amino acids‡) inasmuch as it has been shown that the animal organism can synthesize these groups to a certain extent, but often at a rate insufficient to meet biological needs.

Although the modern concept of biological transmethylations is little more than a decade old, it has already opened up many new vistas in the

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*cf. *Borden's Review of Nutrition Research,* October 1950, "Cholesterol."
†cf. *Borden's Review of Nutrition Research,* Jan. 1950, "Nutrition Highlights—1949."
‡cf. *Borden's Review of Nutrition Research,* Nov. 1950, "Dynamic Interrelationships in Amino Acid Nutrition."

understanding of intermediary metabolism and promises to be of even further significance in contributing toward a more complete understanding of the biological behavior of vitamin B_{12} and the various related anti-anemia factors.

Vitamin B_6 and the D-Amino Acids:

Up until quite recently, nutritionists and biochemists have considered the "unnatural" isomeric forms of the amino acids either as nutritionally nonfunctional or, in some cases, possibly even toxic. During the past two or three years, considerable doubt has been shed on this assumption, culminating in 1950, in the finding that the D-amino acids do enter into metabolic processes in the animal organism in the presence of adequate supplies of vitamin B_6 (in the form of the coenzyme, pyridoxal phosphate). First indications of the biological importance of the D-amino acids came from studies with microorganisms which indicated that certain lactic acid bacteria can utilize these compounds when the growth medium is high in vitamin B_6. Further investigation revealed that the amino acid, D-alanine is actually essential for these bacteria, but that given sufficient B_6 the amino acid could be synthesized by these organisms. In the absence of B_6, D-alanine is required pre-formed in the medium. Thus both a need for a D-amino acid and for B_6 as a coenzyme was established. These studies were extended last year to determine the connection, if any, between vitamin B_6 and the metabolism of D-amino acids in higher animal species such as the rat. Animals on a protein-deficient ration and also deficient in vitamin B_6 were unable to fully utilize dietary nitrogen, whether in the form of natural L-amino acids or unnatural D-amino acids. Administration of L-amino acids to B_6-deficient rats caused a nitrogen loss about equal to the amount of the supplement of L-amino acids given. This loss was eliminated when B_6 was provided. Administration of the D-amino acids to B_6-deficient rats, however, caused a far greater loss of nitrogen, indicating that the unnatural isomers actually depressed the level of nitrogen utilization to about one-third of the normal value. Since the unfavorable effects of the D-amino acids were overcome completely by the simultaneous administration of vitamin B_6, it has been concluded that this vitamin bears some specific relation to the metabolism of D-amino acids in animals, as it does in bacteria.

Evidence from these studies combined with the observation of a widespread occurrence of the enzyme, D-amino acid oxidase in animal tissues, and the occasional occurrence of D-amino acids in various natural products as well as the fact that certain bacteria actually need D-alanine, strongly

suggests a highly important role for these compounds in normal metabolism. What this role may be and the significance of these compounds in terms of nutrition and metabolism remain to be determined.

Nutrition of Cells on a Synthetic Medium:

The study of the nutrition of single tissue cells is one of the most fundamental phases of research in the entire field of nutrition. For more than thirty years experiments have been conducted in an attempt to study the requirements for life and growth of isolated tissues in specially devised culture media outside the living organism. For the most part, the media used to keep such cells alive and growing have been derived, directly or indirectly, from the living organism and have consisted of blood plasma, blood serum, body exudates and extracts of various tissues and organs. However, the complexity and variability of these natural media have made it almost impossible to use them in experiments designed to determine the individual, specific substances which are actually needed by the cells for survival and multiplication. Beginning about 17 years ago, attempts have been made to devise nutritive media composed of chemically known ingredients. But all of these had to be supplemented with serum or other natural materials in order to permit survival and growth of the cells.

Early in 1950, a report was published in which — for the first time — it was announced that a completely synthetic medium (in which all constituents were known chemical entities) has at last been devised which is capable of supporting the growth of living cells outside the body for a prolonged but not indefinite period. The medium was devised with particular reference to amino acids, vitamins, nucleic acids and various accessory growth factors. The optimal concentration of the substances for the maintenance of cell life *in vitro* has been established and a completely synthetic feeding solution has been devised. Although this mixture will not support life indefinitely, it has been found adequate to maintain chick embryo cells for an average period of 4 to 5 weeks. The mixture is being used as a basal synthetic medium for further studies on the unidentified growth-promoting substances known to occur in natural media.

As a result of these studies and of others on nearly-synthetic media, a number of interesting new observations on cell nutrition have already been made. These relate (1) to the significance of essential and non-essential amino acids (all of which have been found necessary for cells); (2) to the fact that *cystine,* a so-called "non-essential" amino acid for both rat and

man, is a key amino acid which is the most essential of all the amino acids for cell growth; (3) to the fact that the D-amino acids are not growth-inhibiting*; (4) to the importance of nucleic acids and derivatives, and of glutamie, etc. Evidence for the significance of ATP†, which is known to activate many metabolic processes, and for the value of the various vitamins has not yet been clearly determined.

Chemistry of Vitamin B_{12}:

Shortly after its discovery, vitamin B_{12} was partially identified in 1949 as a complex molecule whose most significant feature was the presence of cobalt. In September of 1950, further clues to the chemical makeup of this vitamin were forthcoming. Even more unusual than the finding of cobalt in an organic nutrient, these latest findings indicate the presence of cyanide in the B_{12} molecule. The cyano group is described as being bound coordinatively to the cobalt atom in what is known as a cyano-cobalt coordination complex. This complex is not present in the derivative, vitamin B_{12a}, which may be the only distinguishing feature between these two forms of the anti-anemia vitamin. The extreme lack of toxicity of vitamin B_{12} indicates that the cyano group is tightly bound within the coordination complex. Dose levels of 1600 mg. per kg. both intraperitoneally and intravenously in mice produced no deaths or toxic symptoms. This corresponds to a dose level 112,000,000 times the daily human dose of 1 microgram of vitamin B_{12} listed in the U.S. Pharmacopeia.

Vitamin B_{12} has been prepared from vitamin B_{12a} by the addition of cyanide ions to a solution of vitamin B_{12a}. The reaction is believed to involve the replacement of a hydroxo group by a cyano group.

New terminology for this vitamin and its various replacement derivatives has been suggested. The name *cobalamin* designates all the B_{12} molecule except the cyano group. Thus, vitamin B_{12} becomes *cyano-cobalamin* and vitamin B_{12a}, *hydroxo-cobalamin.* Other analogs have been prepared — chloro-cobalamin, cyanato-cobalamin, sulfato-cobalamin, etc. — all of which may be converted to crystalline vitamin B_{12} by reaction with cyanide ions.

Other findings, earlier in the year, reported the production of radioactive ("labelled") vitamin B_{12} by addition of radioactive cobalt to the growth medium of the B_{12} synthesizing microorganism, *Streptomyces griseus.* This product may be expected to facilitate considerably the mapping of the metabolic pathways of vitamin B_{12}.

**cf.* "Vitamin B_6 and the D-Amino Acids," this issue.
†Adenosine-triphosphate.

Folic Acid and Pregnancy:

Studies on nutritional status during pregnancy and its influence on both the course of pregnancy and the condition of the newborn infant have continued during 1950. The results have been largely to extend and confirm findings previously reported which indicate a direct relationship between good pre-natal nutrition and the successful outcome of pregnancy with respect to the condition of both mother and child. An additional report, of purely experimental significance at the present time, indicates a possible specific role for folic acid in the maintenance of pregnancy during the early, embryonic stages — in the laboratory animal. Studies with the folic acid antagonist, 4-amino-pteroylglutamic acid (aminopterin), in rats and mice, and in one case with a dog, indicated that feeding of the anti-folic acid compound caused damage, disintegration or actual resorption of the unborn fetus. When aminopterin was injected early during the course of pregnancy, complete resorption occurred; administration at progressively later intervals caused varying degrees of irreversible damage to the fetus. The dosages used were only slightly toxic to the pregnant animal, causing weight loss and a temporary depletion of the bone marrow.

Use of Amino Acids:

Knowledge concerning the nutritive values of the individual amino acids has increased rapidly in recent years. This has led to the development of practical procedures for the supplementation of human food materials and of animal feeds with essential amino acids to improve nutritive values. Recent investigations, however, indicate that indiscriminate supplementation with amino acids may precipitate a dietary imbalance having dangerous consequences. An example of such a situation has been revealed through recent studies on the niacin-tryptophan relationships in rats. It was noticed that an imbalance of two amino acids, phenylalanine and methionine, caused a nervous condition after 12 weeks. The condition was readily corrected by providing large amounts of both niacin and tryptophan. However, when the injury to the nervous system was allowed to persist beyond a critical interval of time, administration of niacin and tryptophan failed to reverse the damage. These studies constitute a warning against the indiscriminate use of amino acids in human or animal feeding, and emphasize the fact that such use of amino acids be guided by adequate experimental evidence before practical applications are made.

Dietary Interrelationships:

The extreme interdependence of dietary factors has been further demonstrated in numerous investigations during 1950. The interrelationships have become so intricate that it is impossible at the present time to do much more than indicate what they are. For example:

1. The interplay among the various anti-anemia factors (vitamin B_{12}, folic acid, vitamin C, etc.) has been described many times in previous issues of this Review.
2. Similarly, the interplay among the so-called "methylating" factors (choline, methionine, betaine) has been described.
3. Again, the influence of the anti-anemia factors (vitamin B_{12}, folic acid, vitamin C) on the metabolism of the aromatic amino acids, tyrosine and phenylalanine, has been reviewed.
4. Lastly, the combined effect of phenylalanine (an aromatic amino acid) and methionine (a lipotropic factor and "methyl donor") on the tryptophan-niacin metabolic cycle has been indicated.

These are four, separate stories each one of which is composed of many intricate parts which must be fitted together before the story can be considered complete and coherent. As if this in itself were not a challenging task to both nutritionist and biochemist, it now becomes apparent that the four stories themselves are all mutually interlaced, so that: Anti-anemia factors have been shown to function as lipotropic factors (i.e., vitamin B_{12}). Lipotropic factors have been found to be of value in the treatment of certain types of macrocytic anemias in man (i.e. choline). Vitamin C which is essential for the metabolism of the aromatic amino acids can be replaced in this function by folic acid. Pernicious anemia is accompanied by neurological manifestations which do not respond to folic acid therapy, but are cleared up by vitamin B_{12}. An imbalance of the aromatic amino acid, phenylalanine (which requires folic acid for its metabolism) and of the amino acid, methionine (which under some conditions may replace folic acid and B_{12} to some extent) likewise precipitate a neurological condition which responds to combined niacin-tryptophan therapy. Chicks on a folic acid-B_{12}-deficient diet fail to grow; when B_{12} is added, growth is improved, but neurological symptoms develop and are cured by folic acid. Folic acid, B_{12}, choline and methionine are all involved in normal chick growth and feathering; are all essential, to varying degrees and depending on experimental conditions, in preventing chick anemia, perosis and kidney damage. These represent only a few of the numerous interrelations among a selected list of vitamins (folic

acid, B_{12}, vitamin C, choline, niacin, folinic acid) and amino acids (methionine, tryptophan, phenylalanine). The ramifications of these interdependencies are endless; the speculations as to the final significance of all of these interrelationships are similarly endless. The ultimate interpretation of these provocative findings appears to be nowhere yet in sight. Nevertheless, studies of these intricate interrelationships are beginning to bear fruit in terms of a gradual elucidation of the mechanisms whereby the various nutritional factors exert their unique biochemical effects upon the body.

Milk-Borne Immunity to Virus:

More than twenty years ago experimental evidence was obtained indicating the existence of a milk-borne immunity against certain virus diseases. Similar suggestions have been made in recent years. These suggestions have received important confirmation during 1950 in a study made with murine poliomyelitis virus.

At various stages of gestation, 100 pregnant mice were inoculated orally with the murine virus. Eighteen viable litters were born to these inoculated mothers. Half the litter were reared by their own mothers and the other half reared by foster mothers some of which were normal. Six additional litters were obtained from mothers not infected until after parturition; six litters from mothers of known immunity and four litters from normal mothers. All of the animals, both mothers and offspring, were tested for immunity. It was found that the immunity of the young was *directly related to the immunity of the foster mother,* rather than to the immunity of the natural mother. A nursing period of 10 days was sufficient to transfer complete protection against the test dose of murine virus.

Milk obtained from immune mothers was then pooled and tested, *in vitro,* for its capacity to neutralize murine poliomyelitis. While normal mouse milk had no detectable neutralizing power, milk from immune mice reduced the virulence of murine virus when tested in dilutions as high as 1:100, and completely neutralized the virus in a dilution of 1:10. The exact nature of the virucidal factor in immune mouse milk has not yet been determined.